


PATENT APPLICATION
TRANSMITTAL LETTER

Docket Number: 4121-121

I hereby certify that this paper is being deposited this date with the U.S. Postal Service as Express Mail in an envelope addressed to Commissioner for Patents, Washington, D.C., 20231 and Express mailed under the provisions of 37 CFR § 1.10


Blake Crouch

December 8, 2000

Date

EL468271268

Express Mail Label Number

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS:

Transmitted herewith for filing is the patent application of:

Applicants: **VON KNEBEL-DOEBERITZ, Magnus**
KLEIN-BAUERNSCHMITT, Petra
ZUR HAUSEN, Harald
SCHLEHOFER, Jorg

Title: **"USE OF ADENO-ASSOCIATED VIRUSES FOR DECREASING THE
RADIOTHERAPY-INDUCED OR CHEMOTHERAPY-INDUCED
RESISTANCE IN CANCER PATIENTS"**

Filing Date: **December 8, 2000 (Express Mail Filing Under 37 CFR § 1.10)**

NOTE: This application is being filed without an Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicant may secure a filing date of December 8, 2000. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a Declaration and Power of Attorney, Assignment and Small Entity Statement will be filed in the Patent and Trademark Office. The undersigned agent affirmatively states that he has been duly authorized and appointed to file this application on behalf of the applicants and their assignee, Deutsches Krebsforschungszentrum Stiftung Des Öffentlichen Rechts, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Deutsches Krebsforschungszentrum Stiftung Des Öffentlichen Rechts is a small entity under the provisions of 37 CFR § 1.9 and 37 CFR § 1.27. Small entity status is claimed.

Please direct correspondence relating to this application to Steven J. Hultquist, IP/TL, P.O. Box 14329, Research Triangle Park, NC 27709, and direct telephonic communications relating to this application to Steven J. Hultquist at (919) 419-9350.

Enclosed are:

- ☒ Specification, claims, and abstract.
☒ 4 sheet(s) of drawing(s).
☐ An assignment of the invention to _____.
☐ Declaration and power of attorney.
☐ A certified copy of a _____ application.
☐ A verified statement to establish small entity status under 37 CFR § 1.9 and 37 CFR § 1.27.

THE PRIORITY OF GERMAN PATENT APPLICATION NO. 198 25 620.5, FILED JUNE 8, 1998, AS WELL AS INTERNATIONAL APPLICATION PCT/DE99/01711 FILED JUNE 8, 1999 IS HEREBY EXPRESSLY CLAIMED. THIS APPLICATION IS SUBMITTED UNDER 37 CFR 1.53 AND 35 USC 371 BASED ON INTERNATIONAL APPLICATION PCT/DE99/01711 CLAIMING PRIORITY OF GERMAN PATENT APPLICATION NO. 198 25 620.5

09/719336

CALCULATIONS

PTO USE ONLY

- 17.
- ☒
- The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO \$860.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)

\$690.00

No international preliminary examination fee paid to USPTO (37 CFR 1.482)

but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$710.00

Neither international preliminary examination fee (37 CFR 1.482) nor

international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$1,000.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)

and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$ 860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

Claims

Number Filed

Number Extra

Rate

Total Claims 9 -20 =

0

X \$18.00

\$

Independent Claims 2 -3 =

0

X \$80.00

\$

Multiple dependent claim(s) (if applicable) 6

+ \$270.00

\$

270.00

TOTAL OF ABOVE CALCULATIONS =

\$

1130.00

Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).

\$

-565.00

SUBTOTAL =

\$

565.00

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

+

TOTAL NATIONAL FEE =

\$

565.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

\$

+

TOTAL FEE ENCLOSED =

\$

565.00

Amount to be

refunded:

\$

Charged


\$

- a. ☒ A check in the amount of \$ 565.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
Intellectual Property/Technology Law
P. O. Box 14329
Research Triangle Park, NC 27709


Steven J. Hultquist
Registration No. 28,021

09/719336

4121-121

528 Rec'd PCT/PTO 08 DEC 2000

UNITED STATES PATENT APPLICATION

of

**VON KNEBEL-DOEBERITZ, Magnus
KLEIN-BAUERNSCHMITT, Petra
ZUR HAUSEN, Harald
SCHLEHOFER, Jorg**

for

**USE OF ADENO-ASSOCIATED VIRUSES FOR DECREASING THE
RADIOTHERAPY-INDUCED OR CHEMOTHERAPY-INDUCED
RESISTANCE IN CANCER PATIENTS**

K 2687

Use of Adeno-Associated Viruses for Decreasing the
Radiotherapy-Induced or Chemotherapy-Induced Resistance in
Cancer Patients

The invention relates to the use of adeno-associated viruses for decreasing the radiotherapy-induced or chemotherapy-induced resistance in patients who suffer from a cancer which is to be treated by radiotherapy or chemotherapy.

Along with the removal by surgery malignant diseases have been treated by radiotherapy and/or chemotherapy thus far. However, the occurrence of side-effects, in particular the development of resistances, limits the use of cytostatic agents. Because of these side-effects chemotherapeutic agents can only be used to a limited extent. Thus, the dosage of a chemotherapeutic agent can only be a dosage which the patient tolerates. However, in most cases such a dosage only achieves a minor curative effect.

Therefore, it is the object of the present invention to reduce or attenuate the problem of resistance induced by radiotherapy or chemotherapy so as to improve the survival rate of cancer patients after a radiotherapy or chemotherapy. In addition, it shall be possible to reduce the subsequent doses of a radiotherapy or chemotherapy. The responsiveness of tumor cells to radiotherapy or chemotherapy shall also be improved.

This object is achieved by the subject matters defined in the claims.

According to the invention the development of a radiotherapy-induced or chemotherapy-induced resistance in patients suffering from a cancer to be treated by

radiotherapy or chemotherapy shall be reduced by using adeno-associated viruses.

It was found surprisingly that following a treatment with human non-pathogenic adeno-associated viruses (AAV) the tumor cells respond in a better way to a subsequent chemotherapeutic or radiotherapeutic measure.

The AAV virus is a human parvovirus which requires co-infection with a helper virus for a productive infection to take place. AAV infects humans in their infancy and is considered non-pathogenic, since no human disease could be correlated with the AAV infection (Adv. in Vir. Res. 1987, 32, 43-306).

According to the inventors' insight an AAV infection in combination with chemotherapeutic or radiotherapeutic measures increases the efficiency of a conventional therapy and reduces resistances which occur, so that a further therapy can be carried out in a more promising way than possible thus far. Any AAV viruses can be used according to the invention. The AAV-2 virus is used preferably.

In animal experiments carried out with immunodeficient naked mice into which small cell lung carcinoma cells were implanted subcutaneously, it could be shown that an AAV infection which was carried out at the same time as a chemotherapy resulted in a faster recession of the tumors. Relapses occurred after a short time in both groups. However, in the AAV-infected chemotherapeutic group they responded better to another chemotherapy than those of the group only treated by means of chemotherapy. This shows that an AAV infection can reduce or even avoid the development of resistances.

The use of the AAV viruses according to the invention can be made before, at the same time with or after the chemotherapy or radiotherapy. However, it is carried out preferably after

a first chemotherapeutic or radiotherapeutic treatment cycle.

In particular in the case of tumor kinds which *per se* show a poor response to a chemotherapy or radiotherapy it may be indicated to carry out the treatment before or together with the chemotherapy/radiotherapy to increase the efficiency of the therapy. By this the treatment doses can be lowered and therefore the side-effects can be reduced.

The radiotherapy or chemotherapy may be any radiotherapy or chemotherapy which is adapted to the cancer to be treated. Such therapies have been known for years and along with the removal of the tumor by surgery they represent the established method of curing cancer diseases or increasing the life expectancy of a patient by some time. Therefore, a person skilled in the art is perfectly familiar with the measures of a radiotherapy or chemotherapy.

The use of AAV viruses according to the invention can be applied to any cancer kinds, the best success being expectable in connection with colon cancers, pancreatic carcinomas and brain tumors (in particular glioblastomas). The small cell lung carcinoma (SCLC) can preferably be treated therewith.

The application according to the invention is made intravenously, by means of infusions, intratumorally, orally (also by means of inhalations) or cutaneously. In this connection, the virus is formulated in a suitable preparation adapted to the pathway of administration. For an intravenous (also as an infusion) and intratumoral administration it is preferred to provide the virus in a physiological common salt solution, Ringer's solution or PBS solution (phosphate-buffered salt solution), for a cutaneous administration it is preferred to provide it in the form of an ointment, suspension or gel, and for oral administration it is preferred to provide it in the form of an aerosol spray.

Depending on the patient's body weight the virus dose employed is 10^9 - 10^{10} AAV particles/kg body weight.

A pharmaceutical composition is also provided according to the invention which in addition to the chemotherapeutic agent (cytostatic agent) contains adeno-associated viruses, in particular AAV-2. All chemotherapeutic agents (cytostatic agents) common in tumor therapy thus far can be used separately or in combination as a chemotherapeutic agent, e.g. cisplatin, etoposide, methothrexate, doxorubicin, cyclophosphamide, trofosfamide, busulfane, cytarabin, fluorouracil, mercaptopurine, vinblastinesulfate, vincristinesulfate, bleomycinsulfate or mitomycin. Thus, it is preferred for an intravenous (also by means of infusion) and intratumoral administration to provide an injection solution, for a cutaneous administration to provide an ointment, for an oral administration to provide an aerosol spray. As a basis for the preparation of the infusion solution physiological common salt solution, Ringer's solution or PBS each are suitable in pure form or as a mixture. The amount of AAV depends on the patient's weight and is 10^9 - 10^{10} particles/kg body weight. In the pharmaceutical composition it is contained in an amount suitable for an average body weight of 70 kg. The accurate dosage of the pharmaceutical composition according to the invention is determined by a physician and depends on the patient's sex and weight, severity of the disease, kind of administration and planned duration of administration. A composition according to the invention may also contain conventional auxiliary agents. The common auxiliary agents such as carriers, binders, blasting agents, lubricants, solvents, solubilizers, release accelerators, release decelerators, emulsifiers, stabilizers, colorants of the taste correctives may be used as auxiliary agents.

The invention is explained in more detail by means of the attached figures.

Figure 1 shows a diagram of the protocol used for determining AAV-2-mediated drug-sensibilization. The proliferation of the SCLC cell lines after the infection and/or drug treatment was determined by the MTT assay (J. Immunol. Methods 1983, 56, pp. 55-63). The relative proliferation (A/Ao) was calculated by the ratio absorption of AAV-2-infected and/or drug-treated cells (A) to absorption of mock-infected untreated cells (Ao).

Figures 2a+b show the AAV-mediated sensibilization of the SCLC cell lines over cisplatin, the relative proliferation (A/Ao) of the SCLC cell lines, NCI-H209 (figure 2a) and NCI-H446 (figure 2b) following a mock infection (a: PBS alone; b: infected with a heat-inactivated gradient of Ad2-infected cells) or infection with various multiplicities of an infection with AAV-2 with (gray columns) or without (white columns) subsequent treatment with IC50 of cisplatin according to Table 1 (TCID, tissue culture infectious dose).

Figure 3 shows the AAV-mediated sensibilization of tumors in naked mice, derived from NCI-H209 cells (5 mice per group). The dose of cisplatin was 3 mg/kg body weight (weekly administration). The etoposide dose was 7.5 mg/kg body weight (administered three times a week). AAV-2 was administered weekly in a MOI of 10^8 TCID/animal. Arrows show the change of the treatment modalities: - interruption of the drug treatment and AAV-2 infection; + beginning of the treatment and the infection.

The invention is explained in more detail by way of the small cell lung carcinoma (SCLC). This carcinoma type is generally characterized by an initially effective chemotherapy and remission of the tumor. However, almost all of the patients suffer from a relapse which resulted from a resistance of the tumor cells to the first applied chemotherapy (usually cisplatin/etoposide). Therefore, it was tested in a model system with a human small cell lung carcinoma cell line whether an infection with AAV enhances the cytotoxic effect of the chemotherapeutic agents in the

cell culture and in tumors of immunodisturbed mice. It is shown that the AAV infection increases significantly the effectiveness of the chemotherapy of SCLC tumor cells and SCLC tumors.

Example 1

Sensibilization of SCLC cell lines over cisplatin and etoposide by AAV infection

Small cell lung carcinoma cell lines (NCI-H69, NCI-H164, NCI-H209 and NCI-H446) (Cancer Res., 1980, 40, 3502-3507; Cancer Res., 1985, 45, 2913-2923) were cultured in RPMI-1640 medium (Eurobio, Raunheim, Germany). HeLa cells were cultured in DMEN (Eurobio, Raunheim, Germany). All growth media were supplemented with glutamine (Eurobio, 1 %), antibiotics (penicillin and streptomycin) and 10 % heat-inactivated fetal calf serum (PAA; Liz, Austria). The cultures were incubated at 37°C in a damp atmosphere with 5 % CO₂ and tested for mycoplasma contaminations at regular intervals.

The adeno-associated virus type 2 (AAV-2) was replicated in HeLa cells using adenovirus type 2 (Ad-2) as a helper. AAV was purified in a cesium-chloride gradient and titrated as described in J. Gen. Virol., 1994, 74, 2655-2662. The adenovirus type 2 inocula were clarified supernatants of Ad2-infected HeLa cells.

The SCLC cells were suspended in PBS and incubated with purified AAV-2 in the indicated multiplicities of infection (MOI). After 45 min at 37°C, unbound absorbed virus was removed by washing using PBS and the growth medium was supplemented. Either PBS or the heat-inactivated fraction (56°C, 30 min) of a CsCl gradient of Ad-2-infected cells alone was used for the controls (mock infection), the density (1.14 g/cm³) of the AAV-2-containing fraction which was used for the AAV-2 purification being indicated in the

respective experiments. The volume/cell ratio of these experiments was 50 times greater (5 ml/16⁶ cells) than the one used for the AAV infections.

The AAV-2-infected or mock-infected cells were treated with cisplatin (Astra Medica, Frankfurt/Main, Germany) or etoposide (Bristol, Munich, Germany) or both in a ratio [cisplatin : etoposide = 1:2.5], the pharmacons, dissolved in PBS, being added to the medium in the indicated concentration.

The proliferation of the SCLC cells after the infection with AAV-2 and/or treatment with chemotherapeutic agents was determined by the modified MTT test (J. Immunol. Methods, 1983, 56, 55-63). After the infection or mock infection, the cells were placed in plates having 24 wells with a density of 10⁵ cells/well and treated with the chemotherapeutic agents in the indicated concentrations. After six days (NCI-H69, NCI-H446) or eight days (NCI-H146, NCI-H209), MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide; Sigma, Deisenhofen, Germany) was added to the culture up to a final concentration of 0.5 mg/ml. The cultures were then incubated at 37°C for 4 h to permit a reduction of MTT into blue formazan by mitochondrial dehydrogenases (Arch. Biochem. Biophys., 1993, 303, 474-482), which indicates active proliferation of the cells. The cells were centrifuged, washed with PBS, and formazan was solubilized in isopropanol. The precipitated proteins were pelleted by centrifugation (1000 rpm, 15 min) and 200 µl samples of the supernatant were measured to determine the optical density at 540 nm (OD540), OD690 being used as a reference and a Titertek Multiskan plus MKII densitometer being employed (Lab Systems, Finland).

The relative proliferation (A/Ao) was defined as the ratio of the absorption (A) measured in the supernatant of AAV-2-infected and/or pharmacon-treated cells, compared with the absorption measured for the supernatant of the mock-infected cells and untreated control cells (Ao). The IC50 value was

defined as pharmacon concentrations resulting in a 50 % inhibition of proliferation.

The AAV-2-infected cells (MOI values as indicated) or mock-infected cells were placed in plates having 24 wells and treated with cisplatin. After six or eight days, the relative proliferation was determined. In addition, kinetic studies were carried out to determine the optimum treatment modalities for SCLC cells after an AAV infection. It was shown in these investigations that the AAV-mediated sensibilization reached a maximum after one to three hours following the infection. In this investigation the chemotherapeutic agents were administered three hours after the AAV infection. In order to exclude effects which are due to factors still present after the purification with the CsCl gradient and the Ad2 heat inactivation, a control of the mock infection with PBS was carried out in addition to the mock infection with the fraction of the respective gradient of a cell lysate of Ad2-infected cells.

The relative proliferation of NCI-H209-SCLC cells and NCI-H446-SCLC cells was measured after the infection with various multiples of the infectious units (MOI) of AAV-2 (10^1 - 10^6 tissue culture infectious dose (TCID) per cell) with and without subsequent treatment with cisplatin with the IC50 values listed in Table 1.

Table 1

Concentration of the chemotherapeutic agents which result in a 50 % inhibition of the proliferation (IC50) of the SCLC cell lines

NCI-H69	0.2	0.26	0.08/0.2
NCI-H146	0.11	0.025	0.008/0.02
NCI-H209	0.007	0.053	0.006/0.015
NCI-H446	0.15	0.21	0.042/0.105

As shown in Table 1, the SCLC cell lines NCI-H69 and NCI-H446 showed a high intrinsic resistance towards both pharmaceutical preparations, the susceptibility to cisplatin/etoposide treatment being lesser, whereas NCI-H146 cells were highly susceptible to etoposide and the NCI-H209 cells were highly susceptible to both pharmacons.

As follows from figure 2 (a+b), the AAV-2 infection resulted in a decrease of the proliferation rate of the cisplatin-treated cells with a MOI of 10^3 to 10^4 TCID/cell. The infection with a MOI of 10^5 TCID/cell resulted in no further increase. No significant inhibition of the proliferation was observed after the infection with lower MOI values of AAV-2 or after the mock infection, which indicates a specific effect due to the infection with high MOI of AAV-2. The relative proliferation of AAV-2-infected (10^{3-5} AAV/cell) and with cisplatin-treated (IC50) cells was lowered to 0.29 in NCI-H446 cells and to 0.25 in NCI-H209 cells as compared to the relative proliferation in (IC50) cells treated only with cisplatin (0.59 in NCI-H446 cells and 0.5 in NCI-H209 cells).

Example 2

Quantification of the AAV-2-mediated pharmacon sensibilization of SCLC cell lines

In order to quantify the sensitization of cells over chemotherapeutic agents after infection with AAV-2, dose-response curves were prepared. The relative proliferation of the cell lines was determined after a mock infection (PBS) or AAV infection with 10^3 or 10^4 TCID₅₀/cell and subsequent treatment with various concentrations of cisplatin or etoposide or a combination of both pharmaceutical preparations (Table 2).

Table 2

Sensitization factor of SCLC cell lines which were treated with cisplatin and/or etoposide and were infected with AAV.

Cell line	Chemotherapeutic agent	Sensitization factor (A/Ao) * 10 ⁴ Ip. AAV/Cell
NCI-H69	Cisplatin	1.43
	Etoposide	1.30
	Cisplatin/etoposide	1.45
NCI-H146	Cisplatin	1.37
	Etoposide	1.38
	Cisplatin/etoposide	1.33
NCI-H209	Cisplatin	2.33
	Etoposide	2.12
	Cisplatin/etoposide	2.00
NCI-H446	Cisplatin	2.50
	Etoposide	3.00
	Cisplatin/etoposide	3.00

* The relative proliferation (A/Ao) was calculated by the ratio absorption of AAV-2-infected and/or pharmacon-treated (A) cells to absorption of the mock-infected, untreated controls (Ao)

The sensitization factors (SF) were defined as the ratio of the IC₅₀ values of infected cells compared with the IC₅₀ values of mock-infected cells. The sensitization factor

indicates the factor by which the concentration of a chemotherapeutic agent can be reduced after an infection with AAV-2 to obtain the same degree of proliferation inhibition. As summarized in Table 2 the sensibilization by AAV-2 in NCI-H69 and NCI-H146 was moderate (maximum SF about 1.4 with a MOI of 10^4 TCID/cells). The infection of NCI-H209 or NCI-H446 induced a more significant MOI-dependent sensibilization (maximum SF about 3 (NCI-H446) and 2.3 (NCI-H209) with a MOI of 10^5 TCID/cell). The AAV-2-mediated sensibilization did not depend on the chemotherapeutic agent employed.

Example 3

AAV2-mediated pharmacon sensibilization of NCI-H209-derived tumors in naked mice

H209 is a cell line which is derived from a tumor which was not treated chemotherapeutically before cultured and is not resistant to drugs.

Female naked mice (CD1-nu/nu) from Iffa Credo (Brussels, Belgium) were kept in isolators and were given water and food as desired. Experimentally growing SCLC cells (H209) were injected subcutaneously into the side of the six-week-old mice (10^7 cells in 100 μ l PBS per animal). Five months after the inoculation of the cells, when the tumors had reached an average volume of 200 mm³, the animals were infected weekly with AAV-2 (intratumoral injection of 10^8 tissue culture infectious doses (TCID)) and/or with chemotherapeutic agents by intraperitoneal injection of 3 mg/kg body weight cisplatin (weekly) and 7.5 mg/kg body weight etoposide (three times a week). Details of the beginning and end of the treatment are indicated in figure 3. In each group (control, chemotherapeutic treatment, AAV2 infection, treatment + infection) five animals were received. The infected and non-infected animals were kept in separate isolators. The tumor diameters were measured weekly

and the tumor volume was determined by the formula tumor volume = $\frac{1}{2} \times \text{width} \times \text{depth} \times \text{height}$. The relative tumor volume (V/V_0) was determined for each animal and each time (ratio of the tumor volume $[V]$ compared with the tumor at the beginning of the treatment ($[V_0]$).

As follows from figure 3, the treatment with chemotherapeutic agents resulted in a rapid decrease of the tumor volumes and a complete regression after three weeks of treatment. The combination of chemotherapy with AAV2 infection resulted in a more rapid decrease of the tumor volumes compared with animals which had only received a chemotherapy, which indicates a sensibilization of the pharmacon-treated tumor cells by AAV-2. The infection with AAV-2 alone had no significant effect, and the tumor volumes increased to the same extent as did the untreated controls. The treatment was discontinued after complete regression of the tumors and was resumed in the case of a relapse. The treatment of relapses was less effective with animals, which had only received drug treatment, as compared to the animals infected with AAV and treated chemotherapeutically. This shows the development of a resistance to the initial treatment at least in the chemotherapeutically treated animal group. The relapses in AAV-2-treated animals were still susceptible to cisplatin and etoposide treatment but the tumor regression was slower as compared to the regression of the initial tumors. In 3 of 5 AAV-2-infected animals the tumors regressed completely in week 9, in contrast to the tumors of animals which were only treated with chemotherapeutic agents, a complete regression of the tumor not being induced.

Claims

- 1) Use of adeno-associated viruses for lowering the radiotherapy-induced or chemotherapy-induced resistance in patients who suffer from a cancer which is to be treated by radiotherapy or chemotherapy.
- 2) Use according to claim 1, wherein the adeno-associated viruses are used in a dose of 10^9 - 10^{10} AAV particles/kg body weight.
- 3) Use according to any of claims 1 to 2, wherein the adeno-associated virus is adeno-associated virus 2 (AAV-2).
- 4) Use according to any of claims 1 to 3, wherein the cancer to be treated by radiotherapy or chemotherapy is a colon cancer, pancreatic carcinoma or brain tumor or small cell lung carcinoma.
- 5) Use according to any of claims 1 to 4, wherein the use is made intravenously, cutaneously, orally or intratumorally.
- 6) Use according to any of claims 1 to 5, wherein the use is made before, after or simultaneously with a chemotherapy or radiotherapy.

- 7) A pharmaceutical composition containing a chemotherapeutic agent and adeno-associated viruses.
- 8) The pharmaceutical composition according to claim 7, wherein the chemotherapeutic agent is cisplatin and/or etoposide.
- 9) The pharmaceutical composition according to claim 7 or 8, wherein it is an injection or infusion solution, an aerosol spray or an ointment.

ABSTRACT OF THE DISCLOSURE

The invention relates to the use of adeno-associated viruses for decreasing the radiotherapy-induced or chemotherapy-induced resistance in patients who suffer from a cancer which is to be treated by radiotherapy or chemotherapy.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
2210
2211
2212
2213
2214
2215
2216
2217
2218
2219
2220
2221
2222
2223
2224
2225
2226

```
graph TD; A[Cultured cells] --> B[Mock infection]; A --> C[Infection]; B --> D[105 cells / well in 1 ml medium (24 well plates)]; C --> D; D --> E[3 h p.i.: Treatment with chemotherapeutics]; E --> F[Incubation: 6 days (NCI-H69 and NCI-H446) or 8 days (NCI-H146 and NCI-H209)]; F --> G[MTT-assay]; G --> H[A0]; G --> I[A]; H --> J[Calculation of proliferation (A/A0)]; I --> J;
```

The flowchart illustrates the experimental protocol for evaluating the effect of chemotherapeutic agents on HIV-1 infection in cultured cells. The process begins with **Cultured cells**, which are then divided into two groups: **Mock infection** and **Infection**. Both groups are then treated with **10⁵ cells / well in 1 ml medium (24 well plates)**. Following this, the **Infection** group is treated with **3 h p.i.: Treatment with chemotherapeutics**. The cells are then incubated for **6 days (NCI-H69 and NCI-H446) or 8 days (NCI-H146 and NCI-H209)**. After incubation, an **MTT-assay** is performed. The results are then compared between the **A₀** (Mock infection) and **A** (Infection) groups. Finally, the **Calculation of proliferation (A/A₀)** is performed.

Figure 2 a

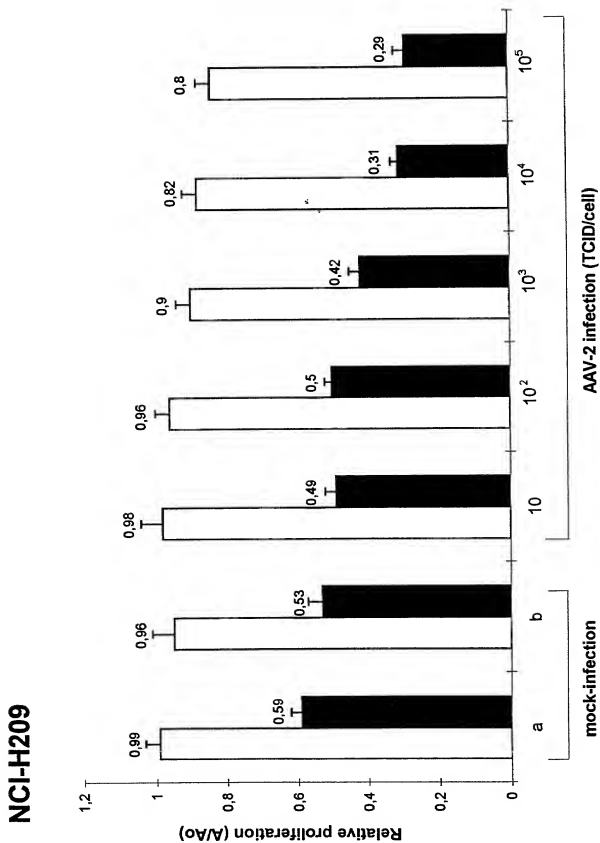


Figure 2 b

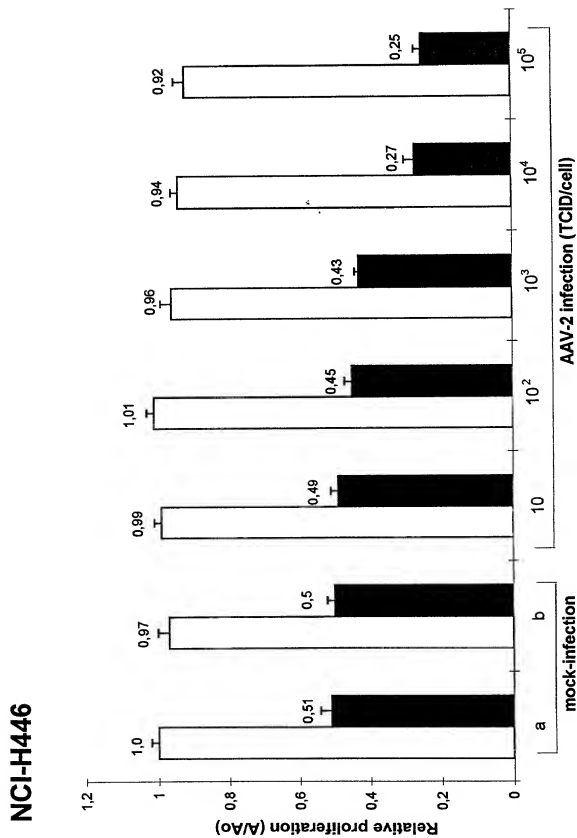
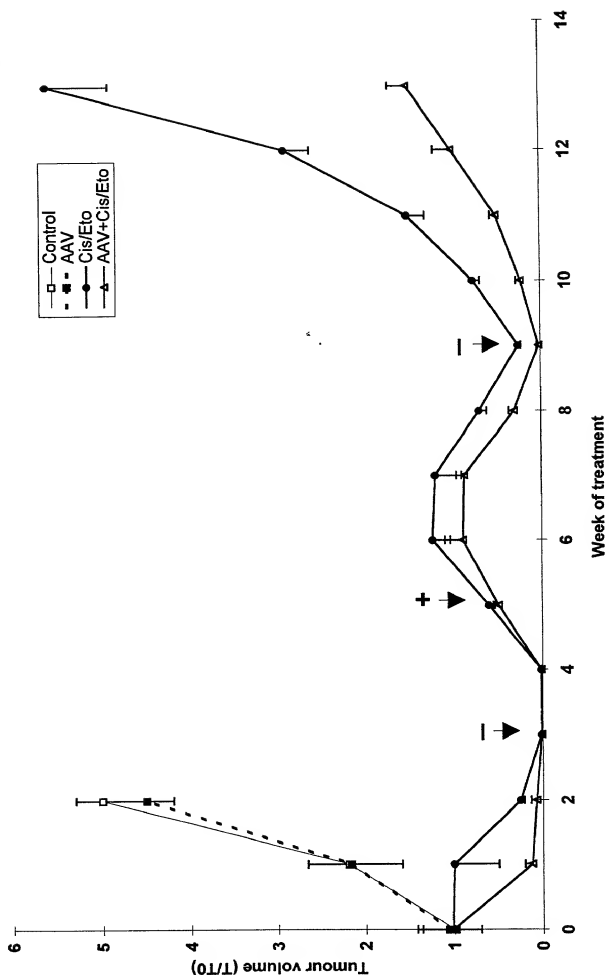


Figure 3





DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name. I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"USE OF ADENO-ASSOCIATED VIRUSES FOR DECREASING THE RADIOTHERAPY-INDUCED OR CHEMOTHERAPY INDUCED RESISTANCE IN CANCER PATIENTS,"** the specification of which was filed on December 8, 2000, claiming the priority of International Patent Application No. PCT/DE99/01711 filed June 8, 1999, and claiming therein the priority of German Patent Application No. 198 25 620.5 filed June 8, 1998.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to the examination and patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PCT/DE99/01711	June 8, 1999	Pending
DE 198 25 620.5	June 8, 1998	Pending
(Application Number)	(Filing Date)	(Status-Patented, Pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

3- STEVEN J. HULTQUIST, REG. NO. 28,021
MARIANNE FUERER, REG. NO. 32,983
WILLIAM A. BARRETT, REG. NO. 42,296

All correspondence in connection with this application should be sent to:

Steven J. Hultquist
Intellectual Property/Technology Law
P. O. Box 14329
Research Triangle Park, NC 27709
Telephone: (919) 419-9350

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full Name of First Inventor: MANGUS VON KNEBEL-DOEBERITZInventor's Signature [Signature]

Date

Feb 10/01/2001

Residence:

Rainweg 93, D-69118 Heidelberg, GERMANY

DEX

Citizenship:

Germany

Post Office Address:

Rainweg 93, D-69118 Heidelberg, GERMANY

2-00 Full Name of Second Inventor: PETRA KLEIN-BAUERNSCHMITTInventor's Signature [Signature]

Date

Feb 19/01/2001

Residence:

Klingenweg 1, D-69118 Heidelberg, GERMANY

DEX

Citizenship:

Germany

Post Office Address:

Klingenweg 1, D-69118 Heidelberg, GERMANY

3-00 Full Name of Third Inventor: HARALD ZUR HAUSENInventor's Signature [Signature]

Date

January 17, 2001

Residence:

Eichenstrasse 1, D-69483 Waldmichelbach, GERMANY

DEX

Citizenship:

Germany

Post Office Address:

Eichenstrasse 1, D-69483 Waldmichelbach, GERMANY

4-00 Full Name of Fourth Inventor: JÖRG SCHLEHOFERInventor's Signature [Signature]

Date

Feb. 08/2001

Residence:

Feilgasse 14 D-69181 Leimen, GERMANY

DEX

Citizenship:

Germany

Post Office Address:

Feilgasse 14 D-69181 Leimen, GERMANY